Acid Production and Curd Toughness in Milks of Different α_{s1}-Casein Types

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Abstract

Skimmilks differing in α_{s1}-casein type were inoculated with starter and renneted. Time to reach pH 5.0 and 4.8 was recorded and toughness of the curd was measured at both pH's. Nine Holstein cows of the following phenotypes were used: 1 A, 3 AB, 3 B, and 2 BC. Numbers were limited by the rarity of the A and C types. Milk from cows of the same type was pooled. The study was repeated eight times over a period of four weeks. Analyses of covariance were done with SNF per cent as the independent variable. The A milk was significantly slower than B and BC in reaching pH 4.8 ($P<.01$). Adjusted means, in minutes, were as follows: BC 289, B 290, AB 304, and A 314. All differences in curd toughness were significant except between AB and B at pH 5.0. The curd was soft when α_{s1}-casein A was involved, while C was associated with toughness. Adjusted means, in grams of force to cut the curd, were: pH 5.0, A 1, AB 19, B 23, BC 119; pH 4.8, A 5, AB 55, B 81, BC 128. The grams of separator slime per 454 g of milk separated also were recorded. Adjusted means were BC .08, B .13, AB .21, A .47. All differences between types were highly significant. Results on a few animals indicate that type of α_{s1}-casein may be of practical importance in some dairy manufacturing operations.

Amino acid composition studies of the genetic variants of α_{s1}-casein indicated that B and C differ only by one amino acid substitution. The rarest type, A, differs from B and C by several amino acids and appears to be slightly lower in molecular weight (3). These differences prompted the speculation that milks of the various genetic types might behave differently under conditions encountered in the manufacture of dairy products. Preliminary studies of some individual cows indicated that milks containing the A type of α_{s1}-casein formed softer curds than others during the renneting process. Ledford, O'Sullivan, and Nath (4) reported recently that α_{s1}-casein in cheese protein is degraded by the action of rennet and microbial enzymes. This study was made to determine if differences in α_{s1}-casein types (A, AB, B, or BC) affected the rate of acid production and curd toughness after rennet treatment.

Experimental Procedure

One of the problems involved in studies of milks differing in α_{s1}-casein type is milk procurement. Of about 3,000 cows studied, only five produced milk containing only the A type of α_{s1}-casein. One of the cows is now at Beltsville, Maryland. At the time this study was planned, there were also three AB and two BC cows available at Beltsville. Most cows are the B type and three of these were selected to be used along with the other six mentioned above. All cows were Holsteins. Table 1 shows the calving dates and genetic types of these cows in the other milk protein systems. The calving dates and the types in the other protein systems for the AB group were considered in selecting the B cows.

The experiment began on June 12, 1966. Evening milk from the individual cows was pooled according to α_{s1}-casein type and cooled to about 5 C. The same procedure was followed the next morning. Morning and evening milks were then pooled by α_{s1}-casein type and mixed thoroughly. Each type of milk was pasteurized at 62.8 C for 30 min and cooled to 37.7 C. A sample of 11.4 kg was put through a sterile separator and the skimmilk was cooled to 5 C or lower in ice water. The separator bowl was thoroughly drained and the slime removed and weighed. The whole milk was estimated for fat by the Babcock method and the skimmilk for total solids by the Mojonnier method.

A 4.54-kg sample of each of the four types of skimmilk was inoculated with 6% starter (a strain of *Streptococcus cremoris*) at 32.2 C. Rennet was added 60 min later at the rate of 1 ml of rennet extract per 454.0 kg of milk. Five samples, one of 700 ml and four of 100 ml each, were then taken from the renneted milk and placed in glass beakers in a 32.2 C water bath.

TABLE 1
Milk protein types and dates of calving for cows used

Cow	α_{s1}-Cn Type	Date of calving	β-Cn	κ-Cn	β-Lg
Marie	A	2-11-66	A^1	A	AB
4234	AB	1-9-66	A^1A^2	A	A
4468	AB	4-17-66	A^1A^2	A	A
4470	AB	6-5-66	A^1A^2	AB	AB
4418	B	4-14-66	A^2	A	AB
4440	B	1-14-66	A^1A^2	A	AB
4452	B	6-2-66	A^2	A	B
T-51	BC	9-24-65	A^2A^3	A	AB
T-67	BC	11-9-65	A^2	A	A

As acid developed and coagulation occurred, pH measurements were made on whey from the largest sample. When the pH reached 5.00 ± 0.05 a penetration test was conducted on the curd in two of the smaller samples. When the pH reached 4.80 ± 0.05 penetration tests were made on the two other small samples. A direct reading, extended scale, curd tension meter was used (1). The meter readings indicated the grams of force necessary to penetrate the curd. Time from addition of starter to attainment of the two pH's also was recorded, as well as initial pH of the skimmilk. These procedures were repeated twice a week for four weeks in succession. The same cows were used throughout except that in the third trial one of the AB cows was inadvertently omitted from the evening sample. Also, in the sixth and seventh trials, one of the AB cows was omitted because of mastitis.

Results and Discussion

The average composition of the milk is shown in Table 2. Each value is a mean calculated from the eight samples processed over the four-week period of the study. Since there were differences in SNF content of the various milks, rate of acid production, curd toughness, and separator slime (Table 3) were analyzed by covariance with SNF per cent as the independent variable. Analysis of covariance was also done on the data on rate of acid production, with original pH as the independent variable. However, original pH had very little effect on the rate of acid production.

The results of the analyses of covariance are shown in Table 3. Duncan's multiple range test was used to determine the significance of differences between the means (adjusted for covariance with % SNF) for the various types of milk. Differences between the four types of milk in the time from addition of rennet to the attainment of pH 5.0 were not statistically significant. However, the α_{s1}-casein A milk took significantly longer to reach pH 4.8 than the other milks ($P<.01$).

The differences in the strength of the curd formed in the four types of milk are particularly striking. At pH 5.0 there were significant differences between the A curd (soft) and all others ($P<.05$); and between the BC curd (hard) and all others ($P<.01$). The adjusted mean penetration readings for AB and B curds were not significantly different. However, by the time pH 4.8 was reached, all types of milk were significantly different from each other in curd toughness ($P<.01$). As in the case of rate of acid production, curd toughness was positively correlated with % SNF ($r = .72$ at both pH's, $P<.01$, $b = 61.0$ at pH 5.0, 85.3 at pH 4.8). It is of interest that rate of acid production and curd toughness were not significantly related (at pH 4.8, $r = -.12$, $P>.05$, $b = -0.4$).

All differences between types in the amount of separator slime per 454 g of milk were also highly significant. This was true both when per cent SNF was taken as the independent variable and when grams of force to penetrate the curd at pH 4.8 was taken as the independent

TABLE 2
Average composition of milk[a]

	α_{s1}-Casein type of milk			
	A	AB	B	BC
Original pH	6.91 ± .10	6.87 ± .10	6.95 ± .12	6.84 ± .11
Per cent SNF (skimmilk)	9.49 ± .14	9.02 ± .18	9.06 ± .10	10.25 ± .05
Per cent fat (whole milk)	4.08 ± .44	3.05 ± .51	3.14 ± .21	3.89 ± .16

[a] Means and standard deviations calculated from eight samples for each type of milk.

TABLE 3
Results of analysis of covariance[a]

Dependent variable	Adjusted means by α_{s1}-casein type[b]			
Minutes to reach pH 5.0	B 260	AB 269	A 284	BC 286
Minutes to reach pH 4.8	BC 289	B 290	AB 304	A 314
Grams of force to penetrate curd at pH 5.0	A 1	AB 19	B 23	BC 119
Grams of force to penetrate curd at pH 4.8	A 4	AB 54	B 80	BC 127
Grams of separator slime/454 g milk separated	BC 0.08	B 0.13	AB 0.21	A 0.47

[a] Per cent SNF of the sample was the independent variable. The means are all adjusted for the effect of the independent variable.

[b] Any two means not underscored by the same line are significantly different. The solid lines indicate the 5% level of probability and dotted lines (alone or in combination with the solid lines) indicate the 1% level. Where underscoring is absent all means are significantly different from each other at the 1% level.

variable. The amount of separator slime per 454 g of milk was negatively correlated with curd toughness ($r = -.76$, $P<.01$). However, the regression coefficient was small ($-.002$).

No final conclusions about the role played by the various α_{s1}-caseins in curd formation can be drawn. As stated, the number of cows available was small and this raises the question of whether the milk samples were truly representative of the four α_{s1}-casein types. However, the differences found are striking and do support previous observations. The data support the hypothesis that milk containing α_{s1}-casein A reaches pH 4.8 slower than α_{s1}-casein B or BC milk after adding standard starter and rennet. The curd formed in A milk was softer than that in B milk and, at pH 4.8 at least, AB milk was intermediate. It is unfortunate that C milk was not available. However, the BC results indicate that α_{s1}-casein C leads to the formation of a tougher curd.

The α_{s1}-casein A milk used in this experiment had the greatest amount of separator slime, as well as the softest curd. A reason for this is not apparent. Could it be that α_{s1}-casein A is in some way less stable than B or C? Or could it cause casein micelles to be less suited to the formation of whatever linkages are involved in curd formation? If so, perhaps some of the unstable complexes of α_{s1}-casein A are thrown off during centrifugation into the so-called separator slime. Only one attempt at gaining an insight into makeup of the slime was made. One per cent solutions of samples of the slime were subjected to vertical polyacrylamide electrophoresis under conditions used routinely for genetic typing of caseins (5). All of the samples revealed significant amounts of α_{s1}- and β-casein which were of the types expected.

Much of what has been said in the last paragraph is speculative. The results of this study, when taken with the observation that α_{s1}-casein is degraded in cheese (4), indicate that further experimentation is needed.

Acknowledgment

The authors gratefully acknowledge the helpful suggestions of Dr. M. P. Thompson regarding this study.

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